

Atty. Docket #: ST 98027

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

INTERNATIONAL APPL. NO.: PCT/FR99/01990 :
INTERNATIONAL FILING DATE: -08/16/99- :
APPLICANT: NEIL ROBERTS ET AL :
SERIAL NO: : **ART UNIT:**
FILED: -HEREWITH- : **EXAMINER:**
FOR: "DEVICE FOR THE RAPID MEASUREMENT :
OF :
ENZYMATIC ACTIVITY" :

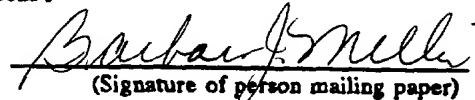
Commissioner for Patents
Box PCT
Washington, D.C. 20231

"Express Mail" No.: EE617838608

Date: -FEBRUARY 15, 2001-

I hereby certify that this paper, along with any other paper or fee referred to in this paper as being transmitted herewith, is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10, postage prepaid, on the date indicated above, addressed to the Commissioner for Patents, Washington, D.C. 20231

-Barbara J. Miller-
(Typed or printed name of mailing paper or fee)


(Signature of person mailing paper)

**TRANSMITTAL OF APPLICATION PAPERS
TO U.S. DESIGNATED/ELECTED OFFICE (DO/EO/US)
CONCERNING A FILING UNDER 35 U.S.C. §371
(37 CFR 1.494 OR 1.495)**

This Transmittal Letter is based upon PTO Form 1390 (as revised in May, 1993).

The above-identified applicant(s) (jointly with their assignee) have filed an International Application under the P.C.T. and hereby submit(s) to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. §371[f]) at any time rather than delay.
4. ☒ A proper Demand for International Preliminary Examination (IPE) was made to the appropriate Authority (IPEA) within the time period required.
5. ☒ A copy of the International Application as filed (35 U.S.C. §371[c][2]) --
- a. ☒ is transmitted herewith (required when not transmitted by International Bureau).
 - b. ☐ has been transmitted by the International Bureau. **See WIPO Publication WO 00/11136.**
 - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A (verified) translation of the International Application into the English language is enclosed -with- Two (2) Sheets of Drawings.
7. ☐ Amendments to the (specification and) claims of the International Application under PCT Article 19 (35 U.S.C. 371[c][3])
- a. ☐ are transmitted herewith (required if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
 - e. ☐ will be submitted with the appropriate surcharge.
8. ☐ A translation of the amendments to the claims (and/or the specification) under PCT Article 19 (35 U.S.C. §371[c][3]) is enclosed or will be submitted with the appropriate surcharge.

International Application No. PCT/FR99/01990

ST 98027

9. ☒ An oath or declaration/power of attorney of the inventor(s) (35 U.S.C. §371(c)[4]) will follow.
☐ and is attached to the translation of (or a copy of) the International Application.
☐ and is attached to the substitute specification.

10. ☒ A translation of at least the Annexes to the IPE Report under PCT Article 36 (35 U.S.C. §371(c)[5]) is enclosed.

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98 is enclosed.
12. ☒ An Assignment for recording and a separate cover sheet in compliance with 37 CFR 3.28 and 3.31 will follow.
13. ☒ A FIRST preliminary amendment is enclosed.
A SECOND or SUBSEQUENT preliminary amendment is enclosed.
14. ☐ A substitute specification (including claims, abstract, drawing) is enclosed.
15. ☐ A change of power of attorney and/or address letter is enclosed.
16. ☒ Other items of information:

- ☒ This application is being filed pursuant to 37 CFR 1.494(c) or 1.495(c), and any missing parts will be filed before expiration of--

☐ 22 months from the priority date under 37 CFR 1.494(c), or

☒ 32 months from the priority date under 37 CFR 1.495(c).

- ☒ The undersigned attorney is authorized by the International applicant and by the inventors to enter the National Phase pursuant to 37 CFR 1.494(c) or 1.495(c).

The following additional information relates to the International Application:

International Application No. PCT/FR99/01990

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- ☒ Receiving Office: France
- ☒ IPEA (if filing under 37 CFR 1.495): EPO
- ☒ Priority Claim(s) (35 USC §§ 119, 365):
FRENCH Appln. 98/10533 filed -August 19, 1998-.
- ☒ A copy of the International Search Report is
- ☐ enclosed.
- ☒ attached to the copy of the International Application.
- ☒ A copy of the Receiving Office Request Form is enclosed.*
- ☒ PCT/IB/304 (1) sheet
- ☒ PCT/IB/308 (1) sheet
- ☒ PCT/IPEA/416 (1) sheet in French
- ☒ PCT/IPEA/409 (6) sheets in French
- ☒ PCT/IPEA/409 (7) pages in ENGLISH
- *☒ PCT/RO/101 (4) pages IN ENGLISH & French

The fee calculation is set forth on the next page of this Transmittal Letter.

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FEE CALCULATION SHEET

☒ A check in payment of the filing fee, calculated as follows, is attached (37 CFR 1.492).

Basic Fee..... \$ 860.00

Total Number of claims in
excess of (20) times \$18..... -0-

Number of independent claims
in excess of (3) times \$80 -0-

Fee for multiple dependent
claims \$270..... -0-

TOTAL FILING FEE... \$ 860.00

Kindly send us the official filing receipt.

The Commissioner is hereby authorized to charge any additional fees which may be required or to credit any overpayment to Deposit Account No. 03-2775. This is a "general authorization" under 37 CFR 1.25(b), except that no automatic debit of the issue upon allowance is authorized. An additional copy of this page is attached.

Respectfully submitted,

By 

William E. McShane
Reg. No. 32,707

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WEM/bjm (5500*81)
Enclosures

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

NEIL ROBERTS, ET AL. : ART UNIT: TBA
SERIAL NO.: TBA : EXAMINER: TBA
FILED: - HEREWITH - : INT'L. APPLN.: PCT/FR99/01990
FOR: **DEVICE FOR THE RAPID MEASUREMENT OF ENZYMATIC ACTIVITY** : INT'L FILING DATE: 8/16/99

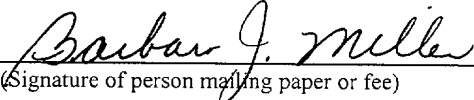
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"Express Mail" No.: EE617838608, Date: February 15, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to Box PCT, Commissioner for Patents, Washington, D.C. 20231.

Barbara J. Miller

(Typed or printed name) of person mailing paper or fee



(Signature of person mailing paper or fee)

PRELIMINARY AMENDMENT

Sir:

Prior to any action on the merits of the accompanying new patent application, kindly
amend the application as follows:

In the Specification:

--Insert the Abstract from PCT/FR99/01990, which is attached hereto as a separate
page.--

In the Claims:

Claim 3, line 1, change "1 or 2" to read --1--;

Claim 5, line 1, change "claims 1 to 4" to read -- claim 1--;

Claim 6, line 1, change "claims 1 to 5" to read -- claim 1--;

Claim 9, line 2, change "claims 1 to 5" to read --claim 1--;

REMARKS

Claims 3, 5-6 and 9 have been amended to refer to only one preceding claim. Each of the dependent claims, as amended, now depends on only one preceding claim. Therefore, no additional fee is required for multiple dependency.

The Abstract from the international application (PCT/FR99/01990) has been inserted on a separate page.

Prompt, favorable action is solicited.

Respectfully submitted,

By



William E. McShane
Registration No. 32,707
P. O. Box 2207
Wilmington, Delaware 19899
(302) 888-6248
Attorney for Applicants

WEM:bjm

::ODMA\MHODMA\CB;131069;1

Abstract

The invention concerns a device for the fast measurement of enzymatic activity in a solid food comprising (i) a container for receiving the sample to be tested; (ii) a reagent particular to the enzyme whereof the activity is to be measured; and (iii) a buffer for placing the enzyme in solution.

1. A device for the fast measurement of enzymatic activity in a solid food, comprising:
(i) a container for receiving the sample to be tested;
(ii) a reagent particular to the enzyme whereof the activity is to be measured;
(iii) a buffer for placing the enzyme in solution.

2/pat

WO 00/11136

PCT/FR99/01990

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DEVICE FOR THE RAPID MEASUREMENT OF ENZYMATIC ACTIVITY

The present invention relates to a device for the rapid measurement of an enzymatic activity in a solid feed, comprising (i) a container designed to contain the test sample, (ii) a reagent specific for the enzyme whose activity it is desired to measure, and (iii) a buffer for dissolving the enzyme.

The feed is preferably a solid feed which is not treated prior to the measurement.

Feeds intended for husbandry animals are usually supplemented with enzymes whose role is mainly to improve the digestibility of the feed ration. These enzymes are usually sprayed in liquid form onto the feeds, in particular as described in patent EP 0,789,291. The enzymes can also be added in powder form to the feed.

Two problems thus arise, the first being to check the uniformity of distribution of the enzymes added to the feed, the second being to quickly and easily evaluate the activity of the enzyme(s) added to the feeds. These problems are raised in particular by feed manufacturers and breeders wishing to check the quality of the feeds they want to give to their animals. Until now, the enzymatic activity could be measured in the laboratory, thus entailing constraints in terms of logistics and delays, these constraints

being a real hindrance when an immediate result is needed.

The present invention satisfies this problem by providing a device for measuring the enzymatic activity of any enzyme-enriched feed intended for animal feed. This device, whose measurement is based on a colorimetric reaction, allows both a qualitative measurement of the enzymatic activity of the test sample and a semi-quantitative measurement of this sample.

Figure 1 represents one embodiment of the invention in the form of a device for measuring enzymatic activity, which is in the form of a column.

The description below can be read with regard to the figure mentioned above.

The device which is the subject of the present invention comprises a container designed to contain the test sample, a reagent specific for the enzyme whose activity it is desired to measure and a buffer for dissolving the said enzyme.

The container of this device can be, without any implied limitation, a column (Figure 1) composed of a graduated narrow bottom part (11) and a wide funnel-shaped top part (12) for introducing various reagents into the column and for mixing them during stirring. The column can also be fitted with a leakproof opening and closure system (13) such as a stopper attached to the body of the column by means of a tab (131).

The container can also consist of a single-use tube (Figure 2).

The container can be made of synthetic material such as a single-use plastic.

5 The container can preferably comprise a cleavable protuberance (14) at its base, allowing the liquid part of its contents to flow out. A constriction (141) retaining the solid morsels of feed is advantageously mounted on the protuberance.

10 Measurement of the enzymatic activity is based on the coloration reaction of the Azo method. The principle of the coloration reaction of the Azo method is based on the enzymatic hydrolysis of a characteristic substrate of an enzyme linked to a
15 chromophore. The reaction produces soluble oligomers which turn the medium blue. The absorbence of the medium can be measured at 590 nm.

 The reagent used in the device is the substrate of the reaction catalysed by the enzyme
20 linked to a chromophore. Thus, the enzymatic hydrolysis reaction releases the chromophoric substrate.

 The device also comprises a buffer for dissolving the enzymes which have been sprayed onto the feed, and for keeping the enzymes at their optimum pH.

25 Mention may be made, by way of example and without any restriction being implied, of the device for demonstrating the activity of xylanases.

To measure the activity of xylanases, the reagent used is "Oat spelt Xylan Remazol Brilliant Blue R" or "Xylazyme AX" (sold by the company Megazyme and consisting of oat or wheat araboxylane linked to a dye).

The buffer used is chosen from acetic acid/sodium acetate; glycine hydrochloride/glycine; aconitic acid/sodium hydroxide; formic acid/sodium formate buffers.

10 Mention may also be made of the device for demonstrating the activity of β -glucanases, which is also based on the coloration reaction of the Azo method.

15 Among the substrates which can be used, mention may be made of 1,3: 1,4- β -D-glucan with Remazol Brilliant Blue R and Beta-Glucazyme sold by the company Megazyme and consisting of beta-glucan combined with azurine.

20 The buffer used is chosen from acetic acid/sodium acetate; glycine hydrochloride/glycine; aconitic acid/sodium hydroxide; formic acid/sodium formate buffers.

25 To measure the activity of cellulase, the substrate used is in the form of Cellazyme lozenges (sold by the company Megazyme). These lozenges consist of substrates based on cellulose and/or on cellulose and xyloglucans polymerized with an azurine dye.

In one preferred embodiment of the present invention, the reagent is in a solid form.

Advantageously, to facilitate the dissolution of the enzyme, a surfactant can be added to the substrate containing the chromophoric agent. This surfactant is chosen in particular from sodium lauryl sulphate and sodium dodecyl sulphate.

According to a better embodiment of the invention, the measurement is carried out in four steps:

- introduction into the container (1) of 10 ml of sample whose enzymatic activity it is desired to measure - for a solid sample, the container should be filled with solid up to the 10 ml graduation mark;
- introduction of the reagent in the form of a solid bead;
- introduction of the specific buffer up to the 20 ml graduation mark;
- after closing the column with the stopper, the column is shaken vigorously several times.

An additional step of separating the liquid phase and the solid phase (by centrifugation or filtration) can optionally be added, to recover the liquid phase and to measure the intensity of the coloration by spectrophotometry or simply by comparison with a colour scale.

The appearance of a blue coloration after a reaction time of 4 to 8 hours confirms the presence of active enzymes, the intensity of the coloration being proportional to the activity of the enzymes present in the sample.

Another advantage of the present invention is the ability to carry out a semi-quantitative measurement of the enzymatic activity. The coloured liquid phase in the column can be recovered by cutting off the cleavable protuberance from the column. The intensity of its coloration can then be compared with an OD calibration curve.

In addition to being fast, the measurement method is very simple and the device can be used anywhere without requiring special equipment. For example, a manufacturer or a breeder can carry out a control measurement as soon as the feed has been manufactured.

The present invention will be described more fully with the aid of the examples which follow, which should not be considered as limiting the invention.

Examples

Two series of tests were carried out on Rovabio xylan LC (mixture of xylanase and beta glucanase from *Penicillium funiculosum*) and on Rovabio xylanase TRLC (xylanase from *Trichoderma reesei*) whose xylanase activity is between 350 and 550 uAXC/ml. It is

estimated that the treatment of spraying the liquid composition on the feeds leads to a level of 70 to 110 uAXC/kg of feed.

The buffer used is the acetate buffer for maintaining a pH of 4.7. The spraying can be carried out on the feed in pulverulent form or in granulated form.

Sample	Activity (before adjust- ment)	Observa- tion at 3 hours	O.D. at 590 nm at 4h30	O.D. at 590 nm at 8h	Observa- tion at 8h
xylanase TRLIC on granules	1336	blue: +++	>3.0	>3.0	blue: +++
xylanase TRLIC on granules	886.7	blue: +	1.567	2.685	blue: ++
xylanase TRLIC on granules	1469.25	blue: +	1.429	2.652	blue: ++
xylanase powder before granula- tion	631.4	no color- ation	0.201	0.666	blue: +

xylanase	1144	blue:	2.309	2.376	blue:
LC on		+++			+++
granules					
xylanase	1386.7	blue: +	1.382	2.484	blue:
LC on					+++
granules					
xylanase	1450.5	blue:	2.872	2.85	blue:
LC on		+++			+++
granules					
xylanase	1330.5	blue: ++	1.233	2.096	blue:
LC on					+++
granules					

CLAIMS

1. Device for measuring the enzymatic activity of a solid feed sample, characterized in that
5 it comprises a container designed to contain the test sample, a reagent specific for the enzyme whose activity it is desired to measure and a buffer for dissolving the said enzyme.

2. Device according to claim 1,
10 characterized in that the test sample is a solid feed, which is preferably untreated.

3. Device according to claim 1 or 2, characterized in that the said container is a single-use graduated column or tube fitted with a leakproof
15 opening and closure system.

4. Device according to claim 3, characterized in that the said container comprises a cleavable protuberance at its base, allowing the liquid part of its contents to flow out.

20 5. Device according to any one of claims 1 to 4, characterized in that the reagent is the substrate for the enzyme linked to a chromophore.

6. Device according to any one of claims 1 to 5, characterized in that the reagent is in solid or
25 liquid form.

7. Device according to claim 1, characterized in that the buffer used to measure the

activity of the enzyme is chosen from acetic acid/sodium acetate; glycine hydrochloride/glycine; aconitic acid/sodium hydroxide; formic acid/sodium formate buffers.

5 8. Use of the device according to claim 1, to measure enzymatic activity quantitatively, characterized in that the coloration obtained is compared with a standard curve.

10 9. Process for measuring the enzymatic activity of a feed, characterized in that 10 ml of sample whose enzymatic activity it is desired to measure are introduced into the device according to claims 1 to 5, reagent in the form of a solid bead is introduced; specific buffer is introduced up to the 15 20 ml graduation mark; after closure of the column with the stopper, the column is shaken vigorously several times; the liquid phase is separated from the solid phase, the liquid phase is recovered and the intensity of the coloration is measured by comparison with a 20 colour scale.

1/2

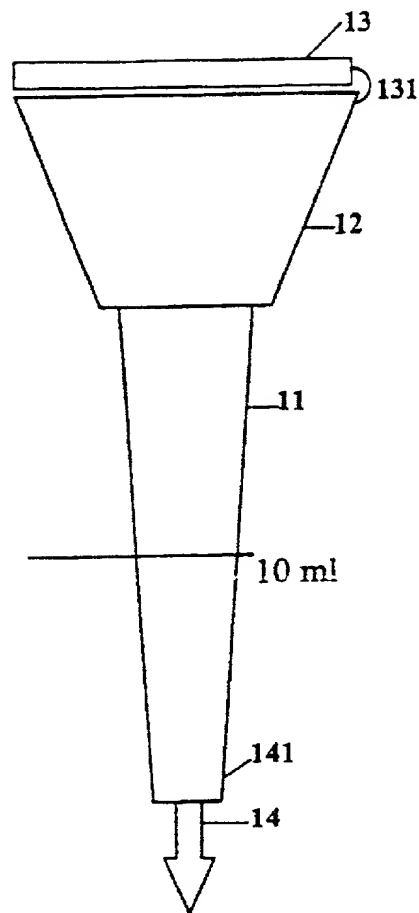


Figure 1

Column of the device for measuring enzymatic activity

2/2

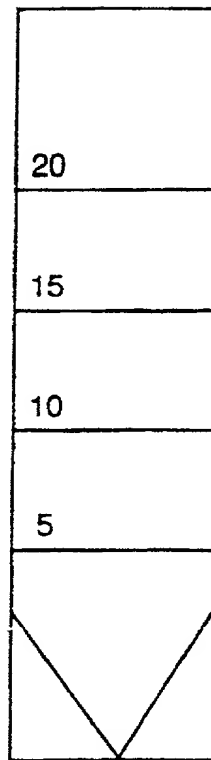


Figure 2

COMBINED DECLARATION AND POWER OF ATTORNEY

Attorney Docket No.

5500*81

ST 98027

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "**Device for the Rapid Measurement of Enzymatic Activity.**"

the specification of which

(check one) ☐ is attached hereto.

☒ was filed on February 15, 2001 as

Application Serial No. 09/763018 and

was amended on February 15, 2001
(if applicable)

was amended through _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)

Priority Claimed

<u>PCT/FR99/01990</u>	<u>International</u>	<u>August 16, 1999</u>	<input checked="" type="checkbox"/> <input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes No
<u>98/10533</u>	<u>France</u>	<u>August 19, 1998</u>	<input checked="" type="checkbox"/> <input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes No
_____	_____	_____	<input type="checkbox"/> <input type="checkbox"/>
(Number)	(Country)	(Day/Month/Year Filed)	Yes No

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application No.) (filing date)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status)
_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status)
		(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

18
In the matter of the above-identified application, please recognize Rudolf E. Hutz, Reg. No. 22,397; John D. Fairchild, Reg. No. 19,756; Harold Pezzner, Reg. No. 22,112; Richard M. Beck, Reg. No. 22,580; Paul E. Crawford, Reg. No. 24,397; Patricia Smink Rogowski, Reg. No. 33,791; Robert G. McMorro, Jr., Reg. No. 30,962; Ashley I. Pezzner, Reg. No. 35,646; William E. McShane, Reg. No. 32,707; Mary W. Bourke, Reg. No. 30,982; Gerard M. O'Rourke, Reg. No. 39,794; James M. Olsen, Reg. No. 40,408; Francis DiGiovanni, Reg. No. 37,310; Eric J. Evain, Reg. No. 42,517; Daniel C. Mulveny, Reg. No. 45,897; Christine M. Hansen, Reg. No. 40,634; Patrick H. Higgins 39,709 and Elliot C. Mendelson (Agent), Reg. No. 42,878, all of P.O. Box 2207, Wilmington, Delaware 19899-2207 as attorneys with full power of substitution to prosecute this application and conduct all business in the Patent and Trademark Office connected therewith.

Send Correspondence To: Connolly Bove Lodge & Hutz LLP P.O. Box 2207 Wilmington, Delaware 19899-2207		Direct Telephone Calls To: (302) 658-9141	
FULL NAME OF SOLE OR FIRST INVENTOR Neil Roberts		INVENTOR'S SIGNATURE <i>Neil Roberts</i>	
DATE 7/23/04/01		CITIZENSHIP Great Britain	
RESIDENCE 15 Newlyn Drive, Bredbury, GB - Stockport, Cheshire SK6 1EF			
POST OFFICE ADDRESS Aventis Animal Nutrition, S.A., 42 Avenue Aristide Briand, 92160, Antony, France			
FULL NAME OF SECOND JOINT INVENTOR IF ANY Janet Moores		INVENTOR'S SIGNATURE <i>J Moores</i>	
DATE X08-05-01		CITIZENSHIP Great Britain	
RESIDENCE 15 Leyfield Avenue, Romiley, Stockport, Cheshire SK6 4AP			
POST OFFICE ADDRESS Aventis Animal Nutrition, S.A., 42 Avenue Aristide Briand, 92160, Antony, France			
FULL NAME OF THIRD JOINT INVENTOR IF ANY		INVENTOR'S SIGNATURE	
DATE		CITIZENSHIP	
RESIDENCE			
POST OFFICE ADDRESS			
FULL NAME OF FOURTH JOINT INVENTOR IF ANY		INVENTOR'S SIGNATURE	
DATE		CITIZENSHIP	
RESIDENCE			
POST OFFICE ADDRESS			
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FULL NAME OF SIXTH JOINT INVENTOR IF ANY		INVENTOR'S SIGNATURE	
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POST OFFICE ADDRESS			
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